

What is claimed is:

1. A method of assaying for protease activity, comprising:
providing a nucleic acid construct having a sequence encoding an amino terminal portion
of a fluorescent reporter fused to a sequence encoding a substrate of a protease
followed by a sequence encoding a carboxy terminal portion of a fluorescent
reporter protein;
expressing a recombinant fluorescent substrate in the presence of the protease;
detecting a change in quenching of fluorescence in the recombinant fluorescent substrate as
an indication of protease activity.
2. The method of claim 1 wherein the presence of a peptide bond between the amino and
carboxyl-terminal fragment of the fluorescent substrate is essential to generate or maintain
fluorescence.
3. The method of claim 1 wherein fluorescence is quenched by cleavage in the protease
substrate sequence.
4. The method of claim 1 wherein the intrinsically fluorescent protein is GFP.
5. The method of claim 1 wherein the protease is introduced by expression from a nucleic
acid construct.
6. A method for identifying a protease that cleaves a target amino acid sequence,
comprising:
providing a nucleic acid construct having a sequence encoding an amino terminal portion
of a fluorescent reporter fused to a sequence encoding a desired substrate target
followed by a sequence encoding a carboxy terminal portion of a fluorescent
reporter protein;
expressing of the recombinant fluorescent substrate in the presence of a plurality of
proteases;

detecting at least one of the plurality of proteases that recognize the target sequence by quenching of the fluorescence of the reporter.

7. The method of claim 6 wherein the fluorescent reporter protein is GFP.

8. A method for determining a substrate recognized by a test protease, comprising:
inserting each of a plurality of fusion nucleic acid sequences encoding a plurality of protease substrate sequences between sequences encoding the amino and carboxyl-terminus of an intrinsically fluorescent protein to form a library of fusion nucleic acids;

expressing the library of fusion nucleic acids to generate a library of recombinant fusion proteins in the presence of the test protease; and

identifying members of the recombinant fusion protein library having quenched fluorescence.

9. The method of claim 8 wherein the fluorescent reporter protein is GFP.

10. A method of assaying proteolytic activity between a protease and a protease substrate sequence of amino acids comprising:

(a) inserting a nucleic acid sequence of amino acids into a surface exposed loop of an intrinsically fluorescent protein to form a recombinant protein;

(b) expressing the recombinant protein substrate;

(c) purifying the recombinant protein substrate; and

(d) detecting quenching of fluorescence in the presence of a protease.

11. The method of claim 10 wherein the fluorescent protein is GFP.

12. A method of assaying proteolytic activity between a protease and a protease substrate sequence of amino acids, comprising:

(a) inserting the protease substrate sequence of amino acids into a surface exposed loop of an intrinsically fluorescent protein to form a recombinant protein;

(b) detecting quenching of fluorescence in the presence of the protease.

13. The method of claim 12 wherein the intrinsically fluorescent protein is GFP.